Use of Hyperbaric Oxygen to Enhance Auricular Composite Graft Survival in the Rabbit Model

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Objective: To evaluate the efficacy of using hyperbaric oxygen to enhance auricular composite graft survival via a prospective, randomized, placebo-controlled, double-blind study.

Design: Eighteen New Zealand White rabbits were randomly assigned to treatment (n=9) and control (n=9) groups after amputation and reattachment of 20 × 10-mm auricular composite grafts. The treatment group received twice-daily hyperbaric oxygen treatments for 5 days. The control group received twice-daily hyperbaric room-air treatments for 5 days. After 21 days, digital photographs of the composite grafts were taken and compared with photographs taken on the day of surgery. From these photographs, digital imaging software was used to calculate the percentage of graft survival.

Results: The treated group (18 ears) had a mean±SD graft survival area of 80.67%±19%, whereas the control group (18 ears) had a mean ± SD graft survival area of 26.33%±29%. Variance analysis with the Snedecor test allowed the comparison of the groups. The paired, 2-tailed t test proved a significant difference (P<.001) between groups.

Conclusion: Hyperbaric oxygen therapy is an effective way to enhance the survival of 2-cm auricular composite grafts.

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Auricular composite grafts have been used for more than 100 years to reconstruct nasal alar defects after tumor ablation. As recounted by Raghavan and Jones,1 auricular composite grafts were first described by Konig in 1902 for use in nasal reconstruction. Forty-one years later, Gillies reintroduced this technique as a means to create an inner vestibular lining when using a forehead flap to reconstruct defects of the lower third of the nose.1 A few years after that, Brown et al described an auricular sandwich graft using a through-and-through auricular composite graft lined on both sides by skin.1

Auricular composite grafts are an excellent means to reconstruct the nose, particularly the nasal alae. They offer a means of maintaining the 3-dimensional structural integrity of the alar region by using autologous tissue. Grafts from the helix have a similar shape and color as the nose; they provide cartilaginous support and ultimately result in low donor-site morbidity.1 The primary limitation of these grafts is their size. Traditionally, the maximum diameter of a full-thickness graft that can be used successfully ranges from 0.75 to 1.5 cm.2-5

Despite anecdotal case reports6,7 regarding the successful use of auricular composite grafts to reconstruct most or all of the nasal alar subunit, there are no quantitative data on the survival rates of large (ie, >1.5-cm) full-thickness grafts. Fear of graft failure may lead surgeons to seek alternative means of reconstructing larger defects. If a large graft is required, it has been the norm in some centers to perform a 2- or 3-stage procedure using a composite graft in conjunction with a paramedian forehead flap in an effort to improve blood supply and, therefore, improve graft survival. The disadvantages of this technique include increased cost due to the need for 2 separate procedures and increased donor-site morbidity due to potential scarring on the forehead or melolabial region. An adjunctive measure to composite grafting of the nasal ala that provides consistently good results would translate into less time spent in the hospital, potentially less cost to the patient, and decreased overall morbidity, while maintaining an excellent cosmetic outcome.

A number of adjunctive measures to composite grafting have already been examined. Of the many pharmacologic agents studied, including nonsteroidal anti-inflammatory drugs, antioxidants, and chlorpromazine hydrochloride, methylprednisolone has shown the most promise in improving graft survival.8,11 Cool-
Hyperbaric oxygen (HBO) therapy also seems to be an effective adjunct to enhance auricular composite graft survival. A number of animal studies have shown that HBO therapy has a role in improving auricular composite graft outcome. Anecdotal reports have shown that HBO therapy also enhances the survival of composite grafts in humans. However, to our knowledge, no study has evaluated the stress effect of placing control groups into a monoplace chamber and increasing the atmospheric pressure, with minimal increase in oxygen delivery. We present a randomized, double-blind, placebo-controlled, prospective animal study that examined the effects of HBO therapy on the survival of full-thickness auricular composite cartilage grafts.

**METHODS**

After receiving approval from the Animal Care Committee at the Massachusetts Eye and Ear Infirmary, Boston, 18 female adult New Zealand White rabbits weighing approximately 3 kg each were transferred to the animal care facility at the Massachusetts Eye and Ear Infirmary. Each animal was treated in accordance with the Animal Welfare Act. After a minimum of 5 days to allow acclimation to the new environment, each animal was anesthetized using a combination of ketamine hydrochloride, 30 mg/kg, and xylazine hydrochloride, 10 mg/kg, administered by intramuscular injection. Each ear was shaved and a 50:50 ratio of intramuscular penicillin G benzathine and penicillin G procaine, 50,000 U/kg, was then administered. Myringotomy was performed with the use of a myringotomy blade (3D Beaver blade; BD, Franklin Lakes, NJ). A prefabricated, semi-circular plastic template measuring 20 mm in diameter was used to mark an area at the lateral base of each ear. A solution of 1% lidocaine with 1:100,000 epinephrine solution was injected into the marked area, and the entire ear was prepped with iodine solution and draped in a sterile fashion. A No. 15 scalpel blade was used to cut out a 20 × 10-mm through-and-through graft. This was immediately placed in sterile isotonic sodium chloride solution and the same procedure was then performed on the opposite ear. Each harvested graft was immediately transplanted into the defect of the opposite ear. A combination of interrupted and running 5-0 chromic, fast-absorbing sutures was used to approximate the skin and perichondrium on the dorsal and ventral surfaces. Bacitracin was then applied to the dorsal and ventral suture lines. The time of the procedure and the order of events were recorded. With the animals still anesthetized, digital pictures were obtained at a set distance, using a metric ruler as a background reference (Figure 1A and Figure 2A).

Within 1 hour of surgery, each animal was placed into an animal research monoplace HBO chamber. Prior to treatment, each animal was randomly assigned to either the treatment or control group by an independent party (n = 9 animals in each group). Depending on group assignment, the animal received either 90-minute treatments of 100% HBO at 2 atm of pressure or 90-minute treatments of medical-grade room air at 1.4 atm of pressure. Each animal was treated twice daily for 5 days. The animals were kept in the animal facility for 21 days after the operation and then humanely killed with the use of a combination of phenytoin sodium and pentobarbital sodium (Butantaniasis solution [D Special; Schering-Plough Animal Health, Union, NJ]), 0.5 mL/kg. Digital images were obtained of the grafts at the same set distance as at the time of surgery (Figures 1B and 2B). All images were given a coded file name and were analyzed by an independent, blinded observer using ImageJ software (freely available at http://rsb.info.nih.gov/ij/), and the percentage of graft survival was calculated from the digital images.

**RESULTS**

To avoid surgical technique variability, all surgical procedures were performed by the same surgeon (D.L.). No animals were dropped from the study. The graft was considered viable when blood flow and hair growth were observed through and along the surface of the graft. The treated group (18 ears) presented a mean ± SD graft survival area of 80.67% ± 19%, whereas the control group (18 ears) had a mean ± SD graft survival area of 26.33% ± 29%. Variance analysis with the Snedecor test allowed the comparison of the groups. t test proved a significant difference (P<.001) between groups.

**COMMENT**

Our results show that HBO therapy is an effective way to enhance auricular composite graft survival, particularly in grafts larger than 1.5 cm. Previous studies have evaluated the effects of HBO therapy on auricular composite graft survival. Although these studies have included placebo control groups, the control animals were...
not subjected to similar experiences within a hyperbaric chamber at an increased pressure. A particular strength of our study was the elimination of this potentially confounding variable by placing all animals in a similarly pressurized system while controlling for the oxygen level specifically.

Li et al\textsuperscript{18} recently performed a similar study that looked at variously sized auricular composite grafts in the rabbit model. Eight animals in their study underwent harvesting of 2-cm circular grafts; 4 of the animals received 7 treatments of HBO at 2.4 atm for 90 minutes and the other 4 received no treatment. The study group had a mean ± SD graft survival rate of 85.8% ± 15.7% and the control group had a graft survival rate of 51.31% ± 38.5% ($P = .048$).

Another strength of our study was the use of semicircular grafts. This shape more closely approximates the clinical use of composite grafts in alar reconstruction compared with circular grafts. Our results support those of Li et al and other previously published data\textsuperscript{14-17} suggesting that HBO therapy is an excellent means of enhancing composite graft survival. The mechanism by which this occurs is likely the result of the hyperoxygenation of tissues and the containment of ischemic injury. Hyperbaric oxygen increases the oxygen-carrying capacity of plasma. By increasing the total oxygen content in the blood, HBO therapy creates a large diffusion gradient that allows oxygen to enter hypoxic tissues.\textsuperscript{18} This is particularly important in poorly vascularized and edematous newly transferred composite grafts, which have an increased oxygen diffusion barrier.

Although hyperoxygenation must play a role in improving graft survival, one school of thought suggests that HBO therapy has its most profound effects on inhibiting ischemia-reperfusion injury.\textsuperscript{20} When a composite graft is harvested, it loses its blood supply until it is successfully sutured in place and reestablishes a blood supply, usually within the first 48 hours. After a period of ischemia, hypoxia and hypoglycemia lead to cellular dysfunction. Once blood flow is reestablished, a second wave of damage is believed to occur as a result of secondary ischemia.\textsuperscript{21} During this period of secondary ischemia, polymorphonuclear cells (PMNs) bind to the endothelium of capillaries within the grafted tissue.\textsuperscript{20,21} Blood flow is thought to be impeded by these PMNs, causing transient hypoxia.\textsuperscript{20-26} Once they migrate into the surrounding tissues, the PMNs release destructive enzymes and trigger reactions, leading to free-radical production and a chain of events that further damages the cells within the graft.\textsuperscript{20,26,27}

A number of studies have looked at the effects of HBO therapy on the events involved in reperfusion injury.\textsuperscript{20} Zamboni et al\textsuperscript{28} demonstrated that HBO therapy decreases PMN adhesion. Their data have been further supported by other studies.\textsuperscript{20,29,30} In addition, data suggest that HBO therapy minimizes free-radical damage.\textsuperscript{20,31} which seems somewhat paradoxical given that HBO results in the generation of free radicals. However, it is believed that these protective effects occur because of HBO inhibiting lipid peroxidation, which thereby minimizes cell damage from free-radical injury.\textsuperscript{31,32} Hyperbaric oxygen therapy also results in increased production of nitric oxide.\textsuperscript{33} At its most basic level, nitric oxide causes vasodilation, which might improve blood flow through the graft. In addition, data indicate that nitric oxide causes vasodilation, which might improve blood flow through the graft. In addition, data indicate that nitric oxide decreases PMN adhesion and may decrease the damaging effects of the superoxide free radical.\textsuperscript{32,34}

Although controversy still exists as to which of these mechanisms has the most profound influence on the healing of ischemic tissues, the clinical effects of HBO on healing are apparent. Case reports have recounted the beneficial effects of HBO therapy on enhancing composite graft survival, and we have found the use of HBO therapy to be helpful in similar cases at our center.\textsuperscript{6,7} As suggested by Li et al,\textsuperscript{18} HBO therapy could potentially act synergistically with corticosteroid therapy and the use of postoperative cooling, and we hope to pursue this hypothesis in future studies.

In conclusion, the present study confirms previously published data that HBO therapy enhances the survival of full-thickness auricular composite grafts. In addition, we have shown that the stress effect of placing animals in a pressurized chamber is not a confounding variable when studying the effects of HBO therapy.
REFERENCES